PATENT
Attorney Docket No. 02508.0120-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
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Reinhold BUCK et al.	)	Group Art Unit: 1797
	)	
Application No.: 10/539,409	)	Examiner: Dirk R. Bass
	)	
Filed: June 17, 2005	)	Confirmation No.: 4997
	)	
For: PERM SELECTIVE ASYMMETRIC HOLLOW FIBRE MEMBRANE FOR THE SEPARATION OF TOXIC MEDIATORS FROM BLOOD	)	

DECLARATION OF HERMANN GOEHL, PURSUANT TO 37 C.F.R. § 1.132

I, Hermann Goehl, Dipl.-Ing. (Univ.) do hereby declare THAT:

1. I am a German citizen, residing at Rosengarten 2, 72406 Bisingen-Zimmern, Germany

2. I have been employed by Gambro Dialysatoren GmbH since 1976. I am currently employed as Consultant for Special Membrane Projects at Gambro Dialysatoren GmbH in Hechingen, Germany.

3. I have carried out research and development on synthetic membranes and dialyzers for different uses during the time of my employment with Gambro Dialysatoren GmbH.

4. I am a co-inventor of the invention set forth in United States Patent Application No. 10/539,409 filed on June 17, 2005 entitled: "Perm selective asymmetric hollow fiber

membrane for the separation of toxic mediators from blood," attached as Exhibit 1 (US 2006/0144782).

5. I am familiar with and have supervised the following experiments directed to the sieving coefficients and physical characteristics of the membranes described below.

Experiment Design

6. We have performed tests to compare

(a) the sieving coefficients of a commercial membrane representing a membrane prepared according to U.S. Patent No. 4,935,141 to Buck et al. ("*Buck*," Exhibit 2) and a high cut-off membrane prepared according to the present invention both *in vitro* and *in vivo*. The results show that the sieving coefficients are different for a number of molecules, which also indicates a difference in the molecular weight cut-off of the membranes;

(b) the pore-sizes on the lumen side of a standard high-flux membrane according to *Buck* and a high cut-off membrane according to the present invention . The results demonstrate physical differences in the selective layer of the membranes; and

(c) by means of electron micrographs the surface on the lumen-side of a standard high-flux membrane according to *Buck*, a high cut-off membrane according to US 2006/0144782, and a plasmafilter membrane. The results highlight the physical differences among the selective layer of each of the membranes.

7. The methods used and the results obtained are described below in more detail.

Methods and Results

8. We have determined the sieving coefficients for a P170H standard high flux membrane, which is commercial membrane prepared according to *Buck* and for a HCO 1100 membrane according to the present invention.

9. The polymer composition of the spinning solution from which the HCO 1100 membrane was prepared consisted of polyethersulfone (wt.-%): 14, PVP K85/90 (wt.-%): 2, PVP K30 (wt.-%): 5, H_2O (wt.-%): 2, NMP (wt.-%): 77. The center fluid for preparing the membrane consisted of H_2O (wt.-%):56, NMP (wt.-%): 44. The temperature of the spinning nozzle was adjusted to 58°C.

10. The polymer composition of the spinning solution from which the P170H membrane was prepared consisted of polyethersulfone (wt.-%): 13.55, polyamide (wt.-%): 0.05, PVP K85/90 (wt.-%): 2, PVP K30 (wt.-%): 5, H_2O (wt.-%): 3, NMP (wt.-%): 76.4. The center fluid for preparing the membrane consisted of H_2O (wt.-%):56, NMP (wt.-%): 44. The temperature of the spinning nozzle was adjusted to 54°C.

11. The preparation of membrane bundles after the spinning process is necessary to prepare the fiber bundle in an adequate way for succeeding performance tests. The first step was to cut the fiber bundles to a defined length of 23 cm, followed by melting the ends of the fibers. Then, the ends of the fiber bundle were transferred into a potting cap. The potting cap was fixed mechanically and a potting tube was put over the potting caps. Afterwards, the potting was done with polyurethane. After the potting the polyurethane was allowed to harden for at least one day. Then, the potted membrane bundle was cut to a defined length in order to open the ends of the fibers, followed by an optic control of the fiber bundle. After the optical control, the membrane bundles were stored dry before they were used for performance tests.

12. The actual measurement of the sieving coefficients for the membranes indicated above was done in accordance with DIN EN1283 (Exhibit 3), which is the European Standard for determining various parameters of medical devices, namely haemodialysers, haemodiafilters etc.. The determination of sieving coefficients is described in section 5.4.2. and the setup is shown in Figure 6. DIN EN1283 corresponds to the International

Standard ISO 8637 (Exhibit 4), see Section 5.6.2 and Figure 5 for the determination of sieving coefficients.

13. The tests were done first with bovine plasma at a maximum blood flow and 20% of blood flow UF. For the HCO 1100 membrane the QB was 400 ml/min, the UF was 80 ml/min. For the P170H membrane the QB was 500 ml/min, the UF was 100 ml/min. The plasma solution was maintained at a temperature of 37°C ± 1°C and pumped under said defined conditions through the testing device. Then, the concentration of the protein in the feed (in), in the retentate (r) and in the filtrate (f) was determined and the sieving coefficient (SC) was then calculated according to the following equation: SC [%] = 2 * c(f) / [c(in)+c(r)] * 100%.

14. Note that, if the concentration of the protein in the filtrate is zero, a sieving coefficient of 0 % is obtained. If the concentration of the protein in the filtrate equals the concentration of the protein in the feed and the retentate, a sieving coefficient of 100 % is obtained.

15. The Sieving Coefficient experiments in aqueous solution of myoglobin and albumin were performed using two different experimental setups with separate solutions. First, the sieving coefficient of myoglobin was determined. Then the sieving coefficient of albumin was determined as described below.

16. The concentration of myoglobin in the PBS buffer was 100 mg/l. The sieving coefficient experiment for myoglobin was run in single pass with testing conditions defined as follows: The intrinsic flow rate (Jv in cm/s) and wall shear rate (γ in s-1) are fixed whereas the blood flow (QB) and filtration rate (UF) were as follows. For the HCO 1100 membrane the QB was 228 ml/min, the UF was 46 ml/min. For the P170H membrane, the QB was 234 ml/min and the UF was 67 ml/min. The shear rate was set to 500 s-1 and the intrinsic flow rate was defined to be 0.38*10-04 cm/s. The first samples were taken after 15 minutes (pool, retentate, and filtrate) and a second time after 60 min. At

the end, the test-bundle was rinsed for some minutes with PBS-buffer, and then the test was stopped.

17. Subsequently, the SC-test of albumin was performed. 60 g of albumin were dissolved in PBS-buffer and the experiment was run re-circulating, the albumin solution being slowly stirred by a magnetic bar stirrer. For the HCO 1100 membrane the QB was 228 ml/min, the UF was 46 ml/min. For the P170H membrane the QB was 234 ml/min and the UF was 67 ml/min. After 15 minutes, the flow was switched to single-pass and samples (pool, retentate, filtrate) were taken.

Table I

Sieving coefficient (%) data		
Filter type	**Plasma[1]**	**Aqueous[2]**
	Validation	Validation/Others
P170H		
Vitamin B12	100	n.d.
Inulin	100	n.d.
beta2M	75	n.d.
Myoglobin	25	70
Albumin	<1	8
HCO 1100		
Vitamin B12	100	n.d.
Inulin	100	n.d.
beta2M	n.d.	n.d.
Myoglobin	95	97-98
Albumin	10	46-53

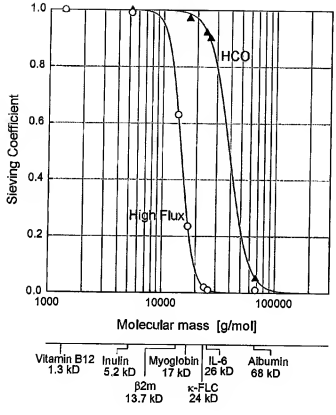
18. Table I depicts the results of the determination of the sieving coefficients for the P170H standard high-flux membrane according to *Buck* as well as for the HCO 1100 membrane according to the present invention . As can be seen, both in plasma and in water the HCO 1100 membrane allows for an increased passage of the molecules chosen. Especially for albumin, it becomes evident that the P170H membrane according to *Buck* is a standard high-flux membrane which does not allow for the passage of albumin, whereas the HCO 1100 membrane according to the invention allows for a certain amount of albumin to pass through the membrane.

19. We have further determined the in vivo sieving coefficients for the P170H standard high flux membrane according to *Buck* and for the HCO 1100 membrane according to the present invention. The membranes are the same as have been used for the determination of sieving coefficients in vitro above.

20. The sieving coefficients have been determined as described in Morgera et al, "Intermittent high permeability haemofiltration in septic patients with acute renal failure," *Intensive Care Med.* 29:1989-1995 (2003) (Exhibit 5).

21. Again, it can be seen in Figure 1 that the high-flux membrane according to *Buck* has a cut-off in the range of between 20 and 30 kD, whereas the HCO 1100 membrane allows for larger middle molecules, such as those having a molecular weight of up to 45,000, to pass the membrane. At the same time, the membrane ensures a significant retention of larger proteins with molecular weights greater than 60 kD, such as clotting factors and immunoglobulins.

Figure 1

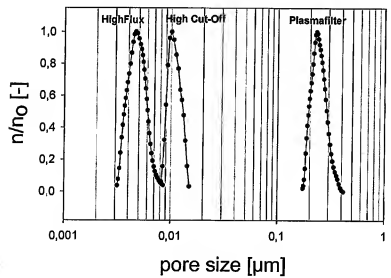


22. We further determined the pore sizes on the lumen side (selective layer) of the P170H standard high flux membrane according to *Buck*, the HCO 1100 membrane according to the present invention and the plasmafilter membrane.

23. The membrane designated "Plasmafilter" in the below text and figures was prepared from a polymer solution consisting of polyethersulfone (wt.-%): 18, PVP K85 (wt.-%): 3.25, PVP K30 (wt.-%): 8, H_2O (wt.-%): 6, NMP (wt.-%): 64.75. The center fluid for preparing the membrane consisted of H_2O (wt.-%): 22, NMP (wt.-%): 78. The temperature of the spinning nozzle was adjusted to 49°C.

24. The tests were performed on our behalf at the laboratories of Gustavo Capanelli, Dipartimento di Chimica e Chimica Industriale, Università di Genova, Genoa, Italy. The method used is generally referred to as the liquid-liquid displacement porosimetry (LLDP). The measurements were done according to Capanelli et al., "Ultrafiltartion characterization methods," *J. Membrane Sci.* 15:289-313 (1982) (Exhibit 6). See also Calvo et al., "Comparison of liquid-liquid displacement porosimetry and scanning electron microscopy image analysis to characterize ultrafiltration track-etched membranes", *J. Membrane Sci.* 239:189-197 (2004) (Exhibit 7) for more insight into LLDP and scanning electron microscopy (see below).

Figure 2



25. Figure 2 demonstrates the physical difference of the HCO 1100 membrane in comparison to the high-flux membrane of the prior art. It further demonstrates the difference to membranes which are used for plasma separation.

26. We further prepared scanning electron micrographs of the lumen side of a P170H standard high flux membrane according to *Buck*, of a HCO 1100 membrane according to the present invention and a Plasmafilter membrane with a Quanta 200 (from FEI) with the following parameters:

Table II

Membrane	Magnification	Pressure	HV	WD
HighFlux	60000	1.52e-4 Pa	24000	4.7 mm
HCO	60000	1.31e-4 Pa	22000	4.5 mm
Plasmafilter	60000	0.12e-4 Pa	24000	5.2 mm

Figure 3

27. Figure 3 further corroborates the results of the LLDP, showing that the inner surface of the HCO 1100 membrane has larger pores with a slightly differing structure on the surface compared to the standard high-flux membrane according to *Buck*. For comparison, the SEM of the Plasmafilter membrane has also been added, which is basically different from both the high-flux and the HCO 1100 membrane. Such plasma separation membrane was also referred to in Example 2 of US 2006/0144762 for comparative purposes.

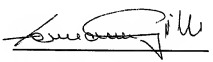
Conclusions

28. The membrane prepared according to *Buck* is different from a membrane of the present invention, at least with regard to its sieving coefficients in plasma and in water, as well as with regard to its physical structure, for example with respect to the inner surface.

29. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

17.08.2010

Date



Hermann GOEHL